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13. ABSTRACT

This report was presented at the Proceedings of the 2nd Annual Conference on Environmental Toxicology, sponsored by the SysteMed Corporation and held in Fairborn, Ohio on 31 August, 1 and 2 September 1971. Major technical areas discussed included toxicological evaluation of volatile halogenated compounds, protection of the public against air pollution and toxicological problems with aircraft, missiles, and space vehicles.

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PAPER NO. 19

ANIMAL PATHOLOGY RESULTING FROM LONG-TERM EXPOSURE TO LOW LEVELS OF MONOMETHYLHYDRAZINE

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INTRODUCTION

Experiments have demonstrated that exposure of monkeys, dogs, rats, and mice to an ambient environment containing monomethylhydrazine (MMH) results in definite dose-related toxic effects (Geiger, 1967; Haun et al., 1968; Haun, 1970; Sopher et al., 1969).

The present experiments were designed to pursue investigation of the toxic effects of intermittent or continuous chronic exposure of monkeys, dogs, rats, and mice to lower levels of environmental MMH.

METHODS

The general experimental procedures and chamber operation techniques are detailed elsewhere in the conference proceedings and in previous publications (Geiger, 1967; Haun et al., 1968; Haun, 1970; Sopher et al., 1969). Briefly, the experimental design involved control animals which were maintained in an ambient environment of atmospheric air for similar periods of time as the experimental animals which were maintained in ambient environments containing various concentrations of MMH either intermittently or continuously for the duration of the exposure period. All animals which received intermittent exposure to MMH were exposed six hours per day and five days per week for the exposure period. At the end of the exposure period, the animals were sacrificed and tissue samples were taken at Wright-Patterson Air Force Base and submitted for pathological examination. Pathological alterations were graded by the degree and distribution of the lesions for each animal and animal group. All exposed animals were compared to appropriate non-exposed control animals. Incidental pathology, not specifically related to the experimental design, was noted; however, the overall occurrence was low and it is not discussed in this presentation.

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RESULTS - I

Experiments 273, 274, and 275 were designed to assess toxic effects resulting from intermittent exposure of monkeys, dogs, rats, and mice to 5 ppm MMH or 2 ppm MMH for a six-month period. Microsections of lungs, hearts, livers, spleens, and kidneys were examined from all large animals and from 10 rats and 10 mice from each experimental group. Microsections of brains and endocrine glands were examined from monkeys and dogs (table I).

TABLE I

ANIMALS EXAMINED FOR PATHOLOGIC CHANGES (SET 1)

Species	Sex	Number of Animals	MMH Exposure Level
Monkey	Female	4	5.0 ppm
Monkey	Female	4	2.0 ppm
Monkey	Female	4	none
Dog	Female	8	5.0 ppm
Dog	Female	8	2.0 ppm
Dog	Female	8	none
Rat	Male	10	5.0 ppm
Rat	Male	10	2.0 ppm
Rat	Male	10	none
Mouse	Male	10	5.0 ppm
Mouse	Male	10	2.0 ppm
Mouse	Male	10	none

Monkeys and Rats

Examination of microsections of tissues from control monkeys and rats, and monkeys and rats exposed to 5 ppm MMH or 2 ppm MMH intermittently for six months reveals that these levels and duration of exposure do not result in experimentally induced histopathological lesions in monkeys and rats at the light microscopic level of examination.

Dogs

Examination of tissues from the exposed dogs reveals that no experimentally induced pathological lesions are produced in lungs, hearts, spleens, brains, or endocrine glands. Uniformly, the dogs exposed to 5 ppm MMH do show periportal hepatic hemosiderosis and cholestasis. Microsections of kidneys from these dogs reveal proximal tubular hemosiderosis. Microsections from dogs exposed to 2 ppm MMH show a similar degree of bile stasis and hepatic and renal tubular hemosiderosis. Morphologically a difference in the degree of toxicity between the 5 ppm MMH and 2 ppm MMH exposure levels, as manifested by bile stasis and hepatic and renal hemosiderosis, cannot be determined by light microscopy.

Mice

Microsections of lungs and hearts of the mice exposed to 5 ppm MMH show no experimentally induced pathological lesions. Microsections of livers of the exposed mice show centrilobular cholestasis, bile duct proliferation, and centrilobular hemosiderosis. Microsections of kidneys and spleens from these animals reveal splenic and renal tubular hemosiderosis of proximal convoluted tubules.

Microsections of lungs and hearts from mice exposed to 2 ppm MMH show no experimentally induced changes. Livers show periportal cholestasis, bile duct proliferation, and hemosiderosis. Microsections of kidneys and spleens from these animals show renal tubular and splenic hemosiderosis but to a lesser degree than was noted in the animals exposed to 5 ppm MMH.

In contrast to dogs at these exposure levels, mice exposed to 2 ppm MMH can be distinguished from mice exposed to 5 ppm MMH by the decreased degree of renal tubular and splenic hemosiderosis induced by the 2 ppm MMH exposure level. The mice also differ from the dogs in that both exposure levels showed bile duct proliferation while this change was not observed in dogs.

RESULTS - II

The second series of experiments were designed to evaluate pathological changes induced by exposure of monkeys, dogs, rats, and mice to lower levels of MMH either continuously or intermittently for six months. Animals submitted for pathologic evaluation from each species were divided into four groups (table II), one of which was a nonexposed control group maintained in a similar chamber for periods of time equal to the exposed periods of experimental groups.

Each species consisted of three exposure groups: (1) exposure to 0.2 ppm MMH continuously for six months; (2) exposure to 1.0 ppm MMH intermittently for a total exposure duration of 144 days; (3) exposure to 0.2 ppm MMH intermittently for a period of 145 days.

TABLE II

ANIMALS EXAMINED FOR PATHOLOGIC CHANGES (SET 2)

Species	Sex	Number of Animals	MMH Exposure Level (ppm)
Monkey	Male	4	none
Monkey	Male	4	0.2 (continuous)
Monkey	Male	4	1.0 (intermittent)*
Monkey	Male	4	0.2 (intermittent)*
Dog	Male	8	none
Dog	Male	8	0.2 (continuous)
Dog	Male	8	1.0 (intermittent)*
Dog	Male	8	0.2 (intermittent)*
Rat	Male	10	none
Rat	Male	10	0.2 (continuous)
Rat	Male	10	1.0 (intermittent)*
Rat	Male	10	0.2 (intermittent)*
Mouse	Female	10	none
Mouse	Female	10	0.2 (continuous)
Mouse	Female	10	1.0 (intermittent)*
Mouse	Female	10	0.2 (intermittent)*

*Exposed for six hours per day for five days per week

Monkeys and Rats

Examination of microsections of tissues from exposed monkeys and rats does not reveal experimentally induced histopathological lesions at either level of exposure whether continuous or intermittent.

Dogs

Examination of lungs, hearts, spleens, kidneys, brains, and endocrine glands of dogs exposed to the three exposure conditions does not reveal experimentally induced changes as compared to nonexposed controls. Examination of livers from dogs exposed, whether continuous or intermittent, shows similar degrees of periportal intracanalicular cholestasis. Hepatic and renal tubular hemosiderosis is not

noted in these animals. Although not available on all animals, lymph nodes show moderate lymphoid hyperplasia of equal degree from the three exposure conditions. Lymphoid hyperplasia is not noted in nonexposed control animals.

Mice

Microsections of lungs and hearts of mice from the three exposure conditions do not show experimentally induced pathological changes. Microsections of livers, spleens, and kidneys of exposed mice show hepatic, splenic, and renal tubular hemosiderosis which is most severe in the animals exposed to 0.2 ppm MMH continuously for a total exposure period of six months and a similar though somewhat less degree of hemosiderosis in animals exposed to 1.0 ppm MMH intermittently over an exposure period of 144 days. Animals exposed to 0.2 ppm MMH intermittently for a total exposure period of 145 days show significantly less hemosiderosis than in the other two exposed groups but significantly more than nonexposed controls. Cholestasis and bile duct proliferation was not noted in mice under these conditions of exposure.

DISCUSSION

These experiments demonstrate that continuous exposure of monkeys or rats at a concentration of 0.2 ppm MMH does not induce histopathological lesions at the light microscopic level. Intermittent exposure of monkeys or rats to MMH concentrations of 5.0 ppm, 2.0 ppm, 1.0 ppm, or 0.2 ppm for the exposure periods of these experiments does not result in experimentally induced pathological lesions.

The same exposure levels and exposure periods do induce pathological lesions in livers and kidneys of dogs and livers, kidneys, and spleens of mice. Under the conditions of these experiments, whether exposed continuously or intermittently, all levels of exposure to MMH (5.0, 2.0, 1.0, and 0.2 ppm) induce periportal hepatic cholestasis in dogs. Intermittent exposure of dogs at the 5.0 ppm MMH and 2.0 ppm MMH levels induces periportal hepatic hemosiderosis and renal proximal tubular hemosiderosis; however, the lower level of exposure (i.e., 0.2 ppm MMH, whether continuous or intermittent) does not induce hepatic and renal tubular hemosiderosis.

Mice show hepatic, splenic, and renal tubular hemosiderosis under all conditions of exposure to MMH; however, the degree of hemosiderosis shows a dose-related pattern. Cholestasis and bile duct proliferation are also dose-related changes and they are not induced by the 1.0 ppm MMH intermittent exposure or by the 0.2 ppm MMH exposure, whether continuous or intermittent.

The fact that the MMH exposure conditions of these experiments induce histopathological changes in dogs and mice but not in monkeys and rats is most probably

explained by species susceptibility to MMH induced hemolysis and species capability for clearing the products of hemolysis. This is confirmed by the differences in histopathological changes induced in dogs and mice by graded decreases in MMH exposure. In dogs, the earliest change appears to be cholestasis, with subsequent hepatic and renal tubular hemosiderosis; whereas in the mice, the initial change appears to be hepatic, splenic, and renal tubular hemosiderosis, with subsequent cholestasis and bile duct proliferation. The changes noted in dogs and mice appear reversible.

Lymphoid hyperplasia was noted in some exposed dogs; however, the limited sampling precludes definitive interpretation of this observation.

The present experiments do not indicate a zero-toxicity MMH exposure level for dogs and mice. The experiments do demonstrate a striking difference in species susceptibility to MMH toxicity, and indicate tissue zero-toxicity levels for monkeys and rats as evaluated by light microscopy.

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DISCUSSION

DR. CARHART (U. S. Naval Research Laboratory): I'd like to ask both the speakers how the concentrations of monomethylhydrazine were monitored.

DR. MAC EWEN (SysteMed Corporation): Perhaps I should let one of our chemists answer that for you. As I indicated, it is a colorimetric method using an AutoAnalyzer on a continuous sampling basis.

MR. VERNOT (SysteMed Corporation): It was an iodometric technique. The monomethylhydrazine reduced the iodine, and the absorbance of the iodine was measured at some wavelength close to the UV. This increased the sensitivity.

DR. DOST (Oregon State University): I would just like to add to Doug's concern about limit values for MMH. We have done some subacute experiments where we intravenously or intraperitoneally infused MMH, and we find that at dose rates of around two thousandths of a micromole or two nanomoles per hour over a 60- to 72-hour period, we can detect a substantial decrease in the capability of rats to oxidize labeled methylamine. If you convert this to inhaled dosage situation, I think, if my calculations are correct, this amounts to something like one microgram per cubic meter, making some assumptions about the ventilation rate of the rat - which makes this a compound of considerable concern in terms of low level, long-term exposure.

LT. COL. MAC KENZIE (USAF School of Aerospace Medicine): I have a loaded question for Dr. Kroe, concerning the bile duct stasis. Do you see this in humans with hemolytic anemias, and do you consider this a part of the hemolytic syndrome?

DR. KROE (Laboratory for Experimental Biology): One can see it in humans with hemolytic anemias, but it would take a pretty severe degree. In addition, we don't that frequently biopsy livers of patients who have intravascular hemolysis. This would tend to be more frequently seen in cases of drug-induced hepatic damage and other forms of direct toxins which would affect the liver directly. I don't know offhand of any direct parallel where humans have been exposed to this type of hemolysis. In the animals (I go back to the comment I made in the presentation), everything we're seeing in the kidneys and livers, and also the spleen of mice, are secondary effects. The target organism is the red blood cell, and the differences we're seeing under the microscope between dogs, rats, mice, and monkeys are very marked differences in species susceptibility to being able to clear the products of hemolysis as well as being susceptible to the hemolytic property of MMH. Certainly from the previous paper it was very well documented that monkeys, for example, showed the effects of MMH, if you're evaluating serum enzymes and the red cells. But, on the other hand, when one looks at the livers, the interpretation that

you must come to is that they're capable of getting rid of the products of hemolysis much more easily. Specifically relating to your question, I can't think of a situation which would be strictly analogous.

DR. BACK (Aerospace Medical Research Laboratory): I have a comment. I'm being deluged with telephone calls from certain cancer research institutes who are interested in monomethylhydrazine, evidently for use therapeutically. I wonder if any of our pathologists have read anything about this on an experimental basis. These are extremely small doses, and I'm wondering if people are beginning to show these kinds of changes. Is anybody familiar with the newer literature on this?

DR. DOST: Part of the interest relates to the hydrazine derivatives, procarbazine particularly, and this is a subject of acute interest in the cancer institute. There have been quite a few studies in the past on carcinogenic and carcinostatic capability of MMH itself and it is still, I think, of questionable effect as a carcinostat, but the derivatives are highly effective in certain types of cancer. Unfortunately, it is a question of curing one shooting with another, you know, because the carcinogenic activity of those derivatives is very high.

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